MycoKeys 66: 1–22 (2020)
doi: 10.3897/mycokeys.66.48389
http://mycokeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
MycoKeys
Launched to accelerate biodiversity research

Ochraceocephala foeniculi gen. et sp. nov., a new pathogen causing crown rot of fennel in Italy

Dalia Aiello[1], Alessandro Vitale[1], Giancarlo Polizzi[1], Hermann Voglmayr[2,3]

1 *Dipartimento di Agricoltura, Alimentazione e Ambiente, sezione Patologia Vegetale, University of Catania, Via S. Sofia 100, 95123 Catania, Italy* **2** *Institute of Forest Entomology, Forest Pathology and Forest Protection, Department of Forest and Soil Sciences, BOKU - University of Natural Resources and Life Sciences, Franz Schwackhöfer Haus, Peter-Jordan-Straße 82/I, 1190 Vienna, Austria* **3** *Division of Systematic and Evolutionary Botany, Department of Botany and Biodiversity Research, University of Vienna, Rennweg 14, 1030 Wien, Austria*

Corresponding author: Dalia Aiello (dalia.aiello@unict.it)

Academic editor: N. Wijayawardene | Received 12 November 2019 | Accepted 17 January 2020 | Published 30 March 2020

Citation: Aiello D, Vitale A, Polizzi G, Voglmayr H (2020) *Ochraceocephala foeniculi* gen. et sp. nov., a new pathogen causing crown rot of fennel in Italy. MycoKeys 66: 1–22. https://doi.org/10.3897/mycokeys.66.48389

Abstract

A new disease of fennel is described from Sicily (southern Italy). Surveys of the disease and sampling were conducted during spring 2017 and 2018 in Adrano and Bronte municipalities (Catania province) where this crop is widely cultivated. Isolations from the margin of symptomatic tissues resulted in fungal colonies with the same morphology. Pathogenicity tests with one isolate of the fungus on 6-month-old plants of fennel reproduced similar symptoms to those observed in nature. Inoculation experiments to assess the susceptibility of six different fennel cultivars to infection by the pathogen showed that the cultivars 'Narciso', 'Apollo', and 'Pompeo' were more susceptible than 'Aurelio', 'Archimede', and 'Pegaso'. Phylogenetic analyses based on a matrix of the internal transcribed spacer (ITS), the large subunit (LSU), and the small subunit (SSU) rDNA regions revealed that the isolates represent a new genus and species within the Leptosphaeriaceae, which is here described as *Ochraceocephala foeniculi* gen. et sp. nov. This study improves the understanding of this new fennel disease, but further studies are needed for planning effective disease management strategies. According to the results of the phylogenetic analyses, *Subplenodomus iridicola* is transferred to the genus *Alloleptosphaeria* and *Acicuseptoria rumicis* to *Paraleptosphaeria*.

Keywords

Fungal disease, Leptosphaeriaceae, pathogenicity, susceptibility

Copyright *Dalia Aiello et al.* This is an open access article distributed under the terms of the Creative Commons Attribution License (CC BY 4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Introduction

Fennel (*Foeniculum vulgare* Mill.), native in arid and semi-arid regions of southern Europe and the Mediterranean area, is used as a vegetable, herb, and seed spice in the food, pharmaceutical, cosmetic, and healthcare industries. Italy is the leading world producer of fennel (around 85% of the world production), with 20,035 ha of area cultivated and a total production of 537,444 tons. Fennel represents an important crop widely cultivated in Sicily (southern Italy) with 1,620 ha harvested and a production of 35,930 tons (ISTAT 2018). Several diseases caused by fungi have been reported from this crop throughout the world (Table 1). Amongst soilborne diseases, brown rot and wilt caused by *Phytophthora megasperma* and crown rot caused by *Didymella glomerata* (syn. *Phoma glomerata*) were reported in Italy (Cacciola et al. 2006; Lahoz et al. 2007).

In 2017, a new disease was first observed on fennel in a farm of Adrano area (Catania province, eastern Sicily, Italy). The disease symptoms were necrotic lesions on the crown, root, and stem of fennel plants. Disease incidence initially was about 5% on 'Apollo' cultivar. However, in 2018 different surveys conducted in the same area showed a high increase of the incidence on three different cultivars with yield losses of about 20–30%. The aims of the present study were to identify the causal agent obtained from symptomatic fennel plants, using morphological characteristics and DNA sequence analyses, to evaluate the pathogenicity of one representative isolate and to evaluate the susceptibility of different cultivars of fennel to the newly described disease.

Materials and methods

Collection of samples and fungal isolates

In order to identify the causal agent of the fennel disease, 30 samples were collected during several surveys in Adrano and Bronte area (Catania province, eastern Sicily). Pieces of tissue obtained from different parts of fennel plants (crown, root, and stem) were surface disinfected for 1 min in 1.5% sodium hypochlorite solution, rinsed in sterile water, placed on potato dextrose agar (3.9% PDA, Oxoid, Basingstoke, UK) amended with 100 mg/L of streptomycin sulfate (Sigma-Aldrich, USA) to prevent bacteria growth, and then incubated at 25 ± 1 °C for seven days. Fungal colonies consistently grown from symptomatic tissues were subcultured on new PDA plates. Subsequently, single-spore isolates were obtained from these pure cultures and stored at –20 °C in sterile 15% glycerol solution. The fungal isolates were provisionally identified by cultural and morphological characteristics, and they were deposited in the culture collection of the Department of Agriculture, Food and Environment, University of Catania. One representative isolate (Di3A-F1; ex holotype culture) was deposited at the Westerdijk Fungal Biodiversity Institute (**CBS**), Utrecht, the Netherlands. The holotype specimen of the new pathogen species was deposited in

Table 1. Main diseases caused by fungal pathogens on fennel.

Disease	Fungal pathogen	Reference
Collar rot	*Sclerotium rolfsii*	Khare et al. 2014
Damping off and Root rot	*Pythium* spp.	Khare et al. 2014; Koike et al. 2015
Vascular wilt	*Fusarium oxysporum*	Shaker and Alhamadany 2015
Vascular wilt	*Verticillium dahliae*	Ghoneem et al. 2009
Root and Foot rot	*Rhizoctonia solani*	Shaker and Alhamadany 2015
Brown rot and Wilt	*Phytophthora megasperma*	Cacciola et al. 2006
Stem rot	*Sclerotinia sclerotiorum*	Choi et al. 2016
Blight and Leaf spot	*Alternaria alternata*	D'Amico et al. 2008
Blight and Leaf spot	*Ascochyta foeniculina*	Khare et al. 2014
Blight and Leaf spot	*Fusoidiella anethi* syn. *Cercospora foeniculi* *Cercosporidium punctum* *Mycosphaerella anethi* *M. foeniculi* *Passalora kirchneri* *P. puncta* *Ramularia foeniculi*	Taubenrauch et al. 2008
Umbel browning and Stem necrosis	*Diaporthe angelicae*	Rodeva and Gabler 2011
Downy mildew	*Plasmopara mei-foeniculi* syn. *P. nivea* sensu lato	Khare et al. 2014
Powdery mildew	*Leveillula languinosa*	Khare et al. 2014
Powdery mildew	*Erysiphe heraclei*	Choi et al. 2015
Leaf spot	*Leptosphaeria purpurea*	Odstrčilová et al. 2002
Leaf spot	*Subplenodomus apiicola* syn. *Phoma apiicola*	Odstrčilová et al. 2002
Leaf spot and blight	*Phoma herbarum*	Shaker and Alhamadany 2015
Crown rot	*Didymella glomerata* syn. *Phoma glomerata*	Lahoz et al. 2007

the fungarium of the Department of Botany and Biodiversity Research, University of Vienna (**WU**).

Morphology

For culture characteristics, cultures were grown on 2% (w/v) malt extract agar (MEA, VWR) and on corn meal agar (CMA, Sigma-Aldrich) supplemented with 2% w/v dextrose (CMD). Colony diameters and morphologies were determined after seven days of incubation at room temperature (22 ± 1 °C) and daylight.

Microscopic observations were made in tap water. Methods of microscopy included stereomicroscopy using a Nikon SMZ 1500 equipped with a Nikon DS-U2 digital camera, and Nomarski differential interference contrast (DIC) using a Zeiss Axio Imager.A1 compound microscope equipped with a Zeiss Axiocam 506 colour digital camera. Images and data were gathered using the NIS-Elements D v. 3.22.15 or Zeiss ZEN Blue Edition software packages. Measurements are reported as maxima and minima in parentheses and the range representing the mean plus and minus the standard deviation of a number of measurements given in parentheses.

DNA extraction and PCR amplification

The extraction of genomic DNA from pure cultures was performed by using the Wizard Genomic DNA Purification Kit (Promega Corporation, WI, USA). Partial regions of six loci (ITS, LSU, and SSU rDNA, *RPB2*, *TEF1*, *TUB2*) were amplified; for details on the primers and annealing temperatures used for PCR and sequencing, see Table 2. The PCR products were sequenced in both directions by Macrogen Inc. (South Korea) or at the Department of Botany and Biodiversity Research, University of Vienna using the ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction Kit v. 3.1 (Applied Biosystems, Warrington, UK) and an automated DNA sequencer (3730xl Genetic Analyser, Applied Biosystems). The DNA sequences generated were assembled with Lasergene SeqMan Pro (DNASTAR, Madison, USA). Sequences generated during the present study were uploaded to Genbank (Table 3).

Phylogenetic analysis

According to the results of BLAST searches in GenBank, the newly generated ITS, LSU, and SSU rDNA sequences of the fennel pathogen were aligned with selected sequences of Leptosphaeriaceae from Gruyter et al. (2013) and complemented with a few recent additions from GenBank. The familial and generic concept of Leptosphaeriaceae implemented here follows the molecular phylogenetic studies of Gruyter et al. (2013), Ariyawansa et al. (2015), and Phookamsak et al. (2019). Due to insufficient *RPB2*, *TEF1*, and *TUB2* sequence data available in Genbank for the study group, the sequences of these markers could not be included in phylogenetic analyses, but they were deposited in GenBank (Table 3). A combined SSU-ITS-LSU rDNA matrix was produced for phylogenetic analyses, with six species of *Coniothyrium* (*C. carteri*, *C. dolichi*, *C. glycines*, *C. multiporum*, *C. telephii*, *C. palmarum*) from Coniothyriaceae added as the outgroup according to the results of the phylogenetic analyses of Gruyter et al. (2013). As the rDNA sequences of the fennel pathogen isolates were (almost) identical (see Results section below), only a single isolate (CBS 145654 = Di3A-F1; ex holotype strain) was included in the final matrix. The GenBank accession numbers of sequences used in the analyses are given in Table 4. Sequence alignments were produced with the server version of MAFFT (http://mafft.cbrc.jp/alignment/server), checked and refined using BioEdit v. 7.2.6 (Hall 1999). The combined data matrix contained 3312 characters; i.e. 607 nucleotides of the ITS, 1333 nucleotides of the LSU and 1372 nucleotides of the SSU).

Maximum likelihood (ML) analyses were performed with RAxML (Stamatakis 2006) as implemented in raxmlGUI 1.3 (Silvestro and Michalak 2012), using the ML + rapid bootstrap setting and the GTRGAMMA substitution model with 1000 bootstrap replicates.

Table 2. Primers used to amplify and sequence the nuclear internal transcribed spacer (ITS), large subunit (LSU) and small subunit (SSU) rDNA regions, the RNA polymerase II second largest subunit (*RPB2*) gene, the translation elongation factor 1-α (*TEF1*) gene and the β-tubulin (*TUB2*) gene.

Gene	Primer	Sequence (5'–3')	Direction	Annealing t (°C)	Reference
ITS	ITS5	GGAAGTAAAAGTCGTAACAAGG	forward	48	White et al. 1990
	ITS4	TCCTCCGCTTATTGATATGC	reverse		White et al. 1990
LSU	LR0R	GTACCCGCTGAACTTAAGC	forward	48	Vilgalys and Hester 1990
	LR5	TCCTGAGGGAAACTTCG	reverse		Vilgalys and Hester 1990
ITS-LSU	V9G	TTAAGTCCCTGCCCTTTGTA	forward	55	Hoog and Gerrits van den Ende 1998
	LR5	TACTTGAAGGAACCCTTACC	reverse		Vilgalys and Hester 1990
	LR2R-A[z]	CAGAGACCGATAGCGCAC	forward		Voglmayr et al. 2012
	LR3[z]	CCGTGTTTCAAGACGGG	reverse		Vilgalys and Hester 1990
	ITS4[z]	TCCTCCGCTTATTGATATGC	reverse		White et al. 1990
SSU	NS1	GTAGTCATATGCTTGTCTC	forward	48	White et al. 1990
	NS4	CTTCCGTCAATTCCTTTAAG	reverse		White et al. 1990
RPB2	RPB2-5F2	GGGGWGAYCAGAAGAAGGC	forward	52	Sung et al. 2007
	RPB2-7cR	CCCATRGCTTGYTTRCCCAT	reverse		Liu et al. 1999
TEF1	EF1-728F	CATCGAGAAGTTCGAGAAGG	forward	52	Carbone and Kohn 1999
	EF1-986R	TACTTGAAGGAACCCTTACC	reverse		Carbone and Kohn 1999
	EF1-728F	CATCGAGAAGTTCGAGAAGG	forward	55	Carbone and Kohn 1999
	TEF1-LLErev	AACTTGCAGGCAATGTGG	reverse		Jaklitsch et al. 2005
	TEF1_INTF[z]	CCGTGAYTTCATCAAGAACATG	forward		Jaklitsch 2009
	TEF1_INT2[z]	CCACTTNGTNGTGTCCATCTTRTT	reverse		Voglmayr and Jaklitsch 2017
TUB2	T1	AACATGCGTGAGATTGTAAGT	forward	52	O'Donnell and Cigelnik 1997
	bt2b	ACCCTCAGTGTAGTGACCCTTGGC	reverse		Glass and Donaldson 1995

[z] internal primers used only for sequencing

Maximum parsimony (MP) bootstrap analyses were performed with PAUP v. 4.0a165 (Swofford 2002). All molecular characters were unordered and given equal weight; analyses were performed with gaps treated as missing data; the COLLAPSE command was set to MINBRLEN. MP bootstrap analyses were performed with 1000 replicates, using 5 rounds of random sequence addition and subsequent TBR branch swapping (MULTREES option in effect, steepest descent option not in effect) during each bootstrap replicate. In the Results and Discussion, bootstrap values below 70 % are considered low, between 70–90 % medium and above 90 % high.

Pathogenicity test

To determine the ability of the representative isolate Di3A-F1 (CBS 145654) to cause disease symptoms, pathogenicity tests were conducted on 6-month-old plants of fennel grown in a growth chamber. Five plants for each of the three replicates were used. The inoculum, which consisted of a 6-mm-diameter mycelial plug from a 10-day-old culture on PDA, was inserted in four points for each crown and the wounds wrapped

Table 3. Characteristics and accession numbers of isolates collected from fennel plants in Sicily.

Strain[1]	Year	Cultivar	Farm	ITS[2]	LSU[2]	SSU[2]	*RPB2*[2]	*TEF1*[2]	*TUB2*[2]
Di3AF1 = CBS 145654*	2017	Apollo	Farm 1	MN516753	MN516774	MN516743	MN520145	MN520149	MN520147
Di3AF2	2017	Apollo	Farm 1	MN516754	MN516775	MN516744			
Di3AF3	2018	Apollo	Farm 1	MN516755	MN516776	MN516745			
Di3AF4	2018	Apollo	Farm 1						
Di3AF5	2018	Apollo	Farm 1	MN516756	MN516777	MN516746			
Di3AF6	2018	Apollo	Farm 1	MN516757	MN516778	MN516747			
Di3AF7	2018	Apollo	Farm 1	MN516758					
Di3AF8	2018	Apollo	Farm 1	MN516759					
Di3AF9	2018	Apollo	Farm 1	MN516760	MN516779	MN516748			
Di3AF10	2018	Apollo	Farm 1	MN516761	MN516780	MN516749	MN520146	MN520150	MN520148
Di3AF11	2018	Apollo	Farm 1	MN516762					
Di3AF12	2018	Apollo	Farm 1	MN516763					
Di3AF13	2018	Apollo	Farm 1	MN516764	MN516781	MN516750			
Di3AF14	2018	Apollo	Farm 1	MN516765	MN516782	MN516751			
Di3AF15	2018	Apollo	Farm 1	MN516766	MN516783	MN516752			
Di3AF16	2018	Apollo	Farm 1	MN516767					
Di3AF17	2018	Apollo	Farm 1	MN516768					
Di3AF18	2018	Narciso	Farm 2						
Di3AF19	2018	Narciso	Farm 2	MN516769					
Di3AF20	2018	Narciso	Farm 2	MN516770					
Di3AF21	2018	Narciso	Farm 2	MN516771					
Di3AF22	2018	Narciso	Farm 2						
Di3AF23	2018	Narciso	Farm 2						
Di3AF24	2018	Narciso	Farm 2						
Di3AF25	2018	Narciso	Farm 2						
Di3AF26	2018	Narciso	Farm 3						
Di3AF27	2018	Narciso	Farm 3						
Di3AF28	2018	Narciso	Farm 3						
Di3AF29	2018	Narciso	Farm 4						
Di3AF30	2018	Narciso	Farm 4	MN516772					
Di3AF31	2018	Narciso	Farm 4						
Di3AF32	2018	Aurelio	Farm 5	MN516773					

Di3A: Cultures stored at the University of Catania, Italy; **CBS**: Culture collection of the Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands. Isolates in bold were sequenced in the present study. [2] **ITS**: internal transcribed spacer rDNA region, **LSU**: large subunit rDNA region, **SSU**: small subunit rDNA region, ***RPB2***: RNA polymerase II second largest subunit gene, ***TEF1***: translation elongation factor 1-α, ***TUB2***: β-tubulin gene.*Ex-type strain.

Table 4. Isolates and accession numbers used in the phylogenetic analyses. Isolate/sequences in bold were isolated/sequenced in the present study.

Taxon	Culture, specimen	Host, substrate	Country	GenBank accession no		
				ITS	LSU	SSU
Alloleptosphaeria iridicola	CBS 143395	*Iris* sp. (Iridaceae)	United Kingdom	MH107919	MH107965	
Alloleptosphaeria italica	MFLUCC 14-934	*Clematis vitalba* (Ranunculaceae)	Italy	KT454722	KT454714	
Alternariaster bidentis	CBS 134021	*Bidens sulphurea* (Asteraceae)	Brazil	KC609333	KC609341	
Alternariaster centaureae-diffusae	MFLUCC 14-0992	*Centaurea diffusa* (Asteraceae)	Russia	KT454723	KT454715	KT454730
Alternariaster helianthi	CBS 119672	*Helianthus* sp. (Asteraceae)	USA	KC609337	KC584368	KC584626
Alternariaster trigonosporus	MFLU 15-2237	*Cirsium* sp. (Asteraceae)	Russia	KY674857	KY674858	
Coniothyrium carteri	CBS 105.91	*Quercus robur* (Fagaceae)	Germany	JF740181	GQ387594	GQ387533
Coniothyrium dolichi	CBS 124140	*Dolichos biforus* (Fabaceae)	India	JF740183	GQ387611	GQ387550
Coniothyrium glycines	CBS 124455	*Glycine max* (Fabaceae)	Zambia	JF740184	GQ387597	GQ387536
Coniothyrium multiporum	CBS 501.91	Unknown	Egypt	JF740186	GU238109	
Coniothyrium palmarum	CBS 400.71	*Chamaerops humilis* (Arecaceae)	Italy	AY720708	EU754153	EU754054
Coniothyrium telephii	CBS 188.71	Air	Finland	JF740188	GQ387599	GQ387538
Heterosporicola chenopodii	CBS 448.68	*Chenopodium album* (Chenopodiaceae)	Netherlands	FJ427023	EU754187	EU754088
Heterosporicola dimorphospora	CBS 165.78	*Chenopodium quinoa* (Chenopodiaceae)	Peru	JF740204	JF740281	JF740098
Leptosphaeria conoidea	CBS 616.75	*Lunaria annua* (Brassicaceae)	Netherlands	JF740201	JF740279	JF740099
Leptosphaeria doliolum	CBS 505.75	*Urtica dioica* (Urticaceae)	Netherlands	JF740205	GQ387576	GQ387515
Leptosphaeria errabunda	CBS 617.75	*Solidago* sp. (hybrid) (Asteraceae)	Netherlands	JF740216	JF740289	
Leptosphaeria macrocapsa	CBS 640.93	*Mercurialis perennis* (Euphorbiaceae)	Netherlands	JF740237	JF740304	
Leptosphaeria pedicularis	CBS 126582	*Gentiana punctata* (Gentianaceae)	Switzerland	JF740223	JF740293	
Leptosphaeria sclerotioides	CBS 144.84	*Medicago sativa* (Fabaceae)	Canada	JF740192	JF740269	
Leptosphaeria slovacica	CBS 389.80	*Balota nigra* (Lamiaceae)	Netherlands	JF740247	JF740315	JF740101
Leptosphaeria sydowii	CBS 385.80	*Senecio jacobaea* (Asteraceae)	UK	JF740244	JF740313	
Leptosphaeria veronicae	CBS 145.84	*Veronica chamaedryoides* (Scrophulariaceae)	Netherlands	JF740254	JF740320	
Neoleptosphaeria rubefaciens	CBS 387.80	*Tilia* (×) *europea* (Malvaceae)	Netherlands	JF740242	JF740311	
Ochraceocephala foeniculi	**Di3AF1 = CBS 145654**	***Foeniculum vulgare* (Apiaceae)**	**Italy**	**MN516753**	**MN516774**	**MN516743**
Paraleptosphaeria dryadis	CBS 643.86	*Dryas octopetala* (Rosaceae)	Switzerland	JF740213	GU301828	
Paraleptosphaeria macrospora	CBS 114198	*Rumex domesticus* (Chenopodiaceae)	Norway	JF740238	JF740305	
Paraleptosphaeria nitschkei	CBS 306.51	*Cirsium spinosissimum* (Asteraceae)	Switzerland	JF740239	JF740308	
Paraleptosphaeria orobanches	CBS 101638	*Epifagus virginiana* (Orobanchaceae)	USA	JF400230	JF740299	
Paraleptosphaeria padi	MFLU 15-2756	*Prunus padus* (Rosaceae)	Russia	KY554203	KY554198	KY554201
Paraleptosphaeria praetermissa	CBS 114591	*Rubus idaeus* (Rosaceae)	Sweden	JF740241	JF740310	
Paraleptosphaeria rubi	MFLUCC 14-0211	*Rubus* sp. (Rosaceae)	Italy	KT454726	KT454718	KT454733

Taxon	Culture, specimen	Host, substrate	Country	GenBank accession no		
				ITS	LSU	SSU
Paraleptosphaeria rumicis	CBS 522.78	*Rumex alpinus* (Polygonaceae)	France	KF251144	KF251648	
Plenodomus agnitus	CBS 121.89	*Eupatorium cannabinum* (Asteraceae)	Netherlands	JF740194	JF740271	
Plenodomus agnitus	CBS 126584	*Eupatorium cannabinum* (Asteraceae)	Netherlands	JF740195	JF740272	
Plenodomus artemisiae	KUMCC 18-0151	*Artemisia* sp. (Asteraceae)	China	MK387920	MK387958	MK387928
Plenodomus biglobosus	CBS 119951	*Brassica rapa* (Brassicaceae)	Netherlands	JF740198	JF740274	JF740102
Plenodomus biglobosus	CBS 127249	*Brassica juncea* (Brassicaceae)	France	JF740199	JF740275	
Plenodomus chrysanthemi	CBS 539.63	*Chrysanthemum* sp. (Asteraceae)	Greece	JF740253	GU238151	GU238230
Plenodomus collinsoniae	CBS 120227	*Vitis coignetiae* (Vitaceae)	Japan	JF740200	JF740276	
Plenodomus confertus	CBS 375.64	*Anacyclus radiatus* (Asteraceae)	Spain	AF439459	JF740277	
Plenodomus congestus	CBS 244.64	*Erigeron canadensis* (Asteraceae)	Spain	AF439460	JF740278	
Plenodomus deqinensis	CGMCC 3.18221	soil	China	KY064027	KY064031	
Plenodomus enteroleucus	CBS 142.84	*Catalpa bignonioides* (Bignoniaceae)	Netherlands	JF740214	JF740287	
Plenodomus enteroleucus	CBS 831.84	*Triticum aestivum* (Poaceae)	Germany	JF740215	JF740288	
Plenodomus fallaciosus	CBS 414.62	*Satureia montana* (Lamiaceae)	France	JF740222	JF740292	
Plenodomus guttulatus	MFLU 15-1876	unidentified dead stem	Germany	KT454721	KT454713	KT454729
Plenodomus hendersoniae	CBS 113702	*Salix cinerea* (Salicaceae)	Sweden	JF740225	JF740295	
Plenodomus hendersoniae	CBS 139.78	*Pyrus malus* (Rosaceae)	Netherlands	JF740226	JF740296	
Plenodomus hendersoniae	LTO	*Salix appendiculata* (Salicaceae)	Austria	MF795790	MF795790	
Plenodomus influorescens	CBS 143.84	*Fraxinus excelsior* (Oleaceae)	Netherlands	JF740228	JF740297	
Plenodomus influorescens	PD 73/1382	*Lilium* sp. (Liliaceae)	Netherlands	JF740229	JF740298	
Plenodomus libanotidis	CBS 113795	*Seseli libanotis* (Apiaceae)	Sweden	JF740231	JF740300	
Plenodomus lijiangensis	KUMCC 18-0186	dead fern fronds	China	MK387921	MK387959	MK387929
Plenodomus lindquistii	CBS 386.80	*Helianthus annuus* (Asteraceae)	former Yugoslavia	JF740232	JF740301	
Plenodomus lindquistii	CBS 381.67	*Helianthus annuus* (Asteraceae)	Canada	JF740233	JF740302	
Plenodomus lingam	CBS 275.63	*Brassica* sp. (Brassicaceae)	UK	JF740234	JF740306	JF740103
Plenodomus lingam	CBS 260.94	*Brassica oleracea* (Brassicaceae)	Netherlands	JF740235	JF740307	
Plenodomus lupini	CBS 248.92	*Lupinus mutabilis* (Fabaceae)	Peru	JF740236	JF740303	
Plenodomus pimpinellae	CBS 101637	*Pimpinella anisum* (Apiaceae)	Israel	JF740240	JF740309	
Plenodomus salviae	MFLUCC 13-0219	*Salvia glutinosa* (Lamiaceae)	Italy	KT454725	KT454717	KT454732
Plenodomus sinensis	MFLU 17-0757	*Plukenetia volubilis* (Euphorbiaceae)	China	MF072722	MF072718	MF072720
Plenodomus tracheiphilus	CBS 551.93	*Citrus limonium* (Rutaceae)	Israel	JF740249	JF740317	JF740104
Plenodomus tracheiphilus	CBS 127250	*Citrus* sp. (Rutaceae)	Italy	JF740250	JF740318	
Plenodomus visci	CBS 122783	*Viscum album* (Viscaceae)	France	JF740256	EU754195	EU754096
Plenodomus wasabiae	CBS 120119	*Wasabia japonica* (Brassicaceae)	Taiwan	JF740257	JF740323	

Taxon	Culture, specimen	Host, substrate	Country	GenBank accession no		
				ITS	LSU	SSU
Plenodomus wasabiae	CBS 120120	*Wasabia japonica* (Brassicaceae)	Taiwan	JF740258	JF740324	
Pseudoleptosphaeria etheridgei	CBS 125980	*Populus tremuloides* (Salicaceae)	Canada	JF740221	JF740291	
Sphaerellopsis filum	CBS 317.68	*Puccinia deschampsiae* uredinium, on *Deschampsia caespitosa*	Germany	KP170657	KP170725	
Sphaerellopsis hakeae	CPC 29566	*Hakea* sp. (Proteaceae)	Australia	KY173466	KY173555	
Sphaerellopsis isthmospora	KUN-HKAS 102225	Unidentified twig	China	MK387925	MK387963	MK387934
Sphaerellopsis macroconidialis	CBS 233.51	*Uromyces caryophylli* on *Dianthus caryophyllus*	Italy	KP170658	KP170726	
Sphaerellopsis paraphysata	CPC 21841	*Pennisetum* sp. (Poaceae)	Brazil	KP170662	KP170729	
Subplenodomus apiicola	CBS 285.72	*Apium graveolens* var. *rapaceum* (Apiaceae)	Germany	JF740196	GU238040	
Subplenodomus drobnjacensis	CBS 269.92	*Eustoma exaltatum* (Gentianaceae)	Netherlands	JF740211	JF740285	JF740100
Subplenodomus valerianae	CBS 630.68	*Valeriana phu* (Valerianaceae)	Netherlands	JF740251	GU238150	
Subplenodomus violicola	CBS 306.68	*Viola tricolor* (Violaceae)	Netherlands	FJ427083	GU238156	GU238231

with Parafilm to prevent desiccation. Fennel plants inoculated with sterile PDA plugs served as a control. After inoculation, plants were covered with a plastic bag for 48 h and maintained at 25 ± 1 °C and 95% relative humidity (RH) under a 12 h fluorescent light/dark regime. Five days after inoculation the presence of a lesion was evaluated in each inoculation point. To fulfill Koch's postulates, symptomatic tissues taken from the crown of each inoculated plant were plated on PDA and the identity of the fungal isolates was confirmed as described above.

Cultivar susceptibility

To evaluate the susceptibility of six different cultivars of fennel to infection by the pathogen, one experiment was conducted on 1 to 2-month-old seedlings of fennel in a growth chamber. Eight plants for each of three replicates were used. The inoculum, which consisted of a 6-mm-diameter mycelial plug from a 10-day-old culture on PDA, was inserted at the crown of each plant and wrapped with Parafilm to prevent desiccation. Fennel plants inoculated with sterile PDA plugs served as a control. All the replicates were enclosed in plastic bags and maintained at 25 ± 1 °C and 95% relative humidity (RH) under a 12 h fluorescent light/dark regime in a growth chamber until the symptoms were observed. Plant mortality (PM), disease incidence (DI) and symptom severity (SS) were evaluated. Symptom severity was rated using a category scale from 0 to 5, where 0 = healthy plant; 1 = necrotic lesion on crown from 0.1 to 0.2 cm; 2 = from 0.3 to 1 cm; 3 = from 1.1 to 2 cm; 4 = from 2.1 to 3.5 cm; 5 = dead plant. The experiment was performed twice.

Statistical analysis

Data about disease susceptibility of examined fennel cultivars from the repeated experiments were analysed by using the Statistica package software (v. 10; Statsoft Inc., Tulsa, OK, USA). The arithmetic means of PM, DI, and SS were calculated, averaging the values determined for the single replicates of each treatment. Percentage data concerning PM and DI were transformed into the arcsine ($\sin^{-1}$ square rootx) prior to analysis of variance (ANOVA), whereas SS values were not transformed. Initial analyses of PM and DI were performed by calculating F and P values associated to evaluate whether the effects of single factor (cultivar) and cultivar × trial interactions are significant. In the post hoc analyses, the corresponding mean values of PM and DI were subsequently separated by the Fisher's least significant difference test ($P = 0.05$). Because ordinal scales were adopted for SS data calculation, different nonparametric approaches were used. Kendall's coefficient of concordance (W) was calculated to assess whether the rankings of the SS scores among fennel cultivars are similar within each trial (cultivar × trial interactions). Since in the susceptibility experiment W was higher than 0.9, the SS scores were at first analysed by using Friedman's nonparametric rank test, and subsequently followed by the all possible pairwise performed with the Wil-

coxon signed-rank at $P < 0.05$. On the other hand, when only the cultivar effects were examined, the Kruskal-Wallis non parametric one-way test was preliminarily applied, calculating χ^2 and P value associated.

Results

Collection of samples and isolates

Symptoms referable to infection (Fig. 1a, b) were detected in five commercial farms surveyed in eastern Sicily, Italy. The disease was observed on 3 different cultivars of fennel (4 to 6-month-old) in open fields. The symptoms consisted of depressed necrotic lesions formed near the soil line and affected crown, root, and stem. The lesion was first light brown with wet appearance, becoming dark brown to black with age and sometimes appearing dry. Under favourable conditions (high humidity), the lesion extended and the infection resulted in a crown and root rot. Fungal colonies representing the new fennel pathogen were consistently obtained from symptomatic tissues. A total of 32 single-spore isolates were collected (Table 3). Preliminary identity of the fungal isolates was based on cultural and morphological characteristics. Among these, 17 isolates were obtained from 'Apollo', 14 from 'Narciso', and one from 'Aurelio' cultivars.

Sequencing

All strains of the new fennel pathogen sequenced had identical LSU, SSU, *RPB2*, *TEF1*, and *TUB2* sequences. Also all ITS sequences were identical, except for a single nucleotide polymorphism (A/G) towards the end of the ITS2 region. All sequences generated during this study were deposited at GenBank; for GenBank accession numbers, see Table 3.

Phylogenetic analyses

Of the 3312 characters included in the phylogenetic analyses, 294 were parsimony informative (222 from the ITS, 62 from the LSU, 10 from the SSU). The best ML tree (lnL = –14211.5558) revealed by RAxML is shown in Figure 2. In the phylogenetic tree, the Leptosphaeriaceae received high (96% ML and MP) support. Within Leptosphaeriaceae, most of the deeper nodes of the tree backbone received low to insignificant support. Highly supported genera include *Alloleptosphaeria*, *Heterosporicola*, *Leptosphaeria* (all three with maximum support) and *Alternariaster* (99% ML and 100% MP), while *Sphaerellopsis* received low (53%) and *Paraleptosphaeria* medium (75%) support only in the ML analyses, and *Plenodomus* and *Subplenodomus* were unsupported. *Subplenodomus iridicola* was not contained within the *Subplenodomus* clade, but sister species to *Alloleptosphaeria italica* with maximum support, and *Aci-*

Figure 1. Symptoms caused by *Ochraceocephala foeniculi* on fennel plants. **a, b** Necrotic lesions and crown rot on 'Narciso' cultivar. **c, d** Necrotic lesions and crown rot on 'Apollo' cultivar. **e** Symptoms on artificially inoculated seedlings of 'Pompeo' cultivar.

cuseptoria rumicis was embedded within the *Paraleptosphaeria* clade, indicating that they are generically misplaced. The new fennel pathogen was placed basal to the *Plenodomus* clade, however, without significant support. Although the new fennel pathogen is closely related to the genus *Plenodomus*, it is morphologically highly distinct. As no suitable described genus is available, a new genus is therefore established here.

Taxonomy

Ochraceocephala **Voglmayr & Aiello, gen. nov.**
MycoBank No: 833933

Etymology. referring to the ochraceous conidial capitula of the type species.

Conidiophores erect, variable in shape and branching, from unbranched, loosely to densely branched up to several times; branching commonly irregularly verticillate. Phialides arising singly or in irregular whorls, cylindrical, lageniform or ampulliform, producing basipetal conidial chains. Conidia in chains, unicellular, thick-walled.

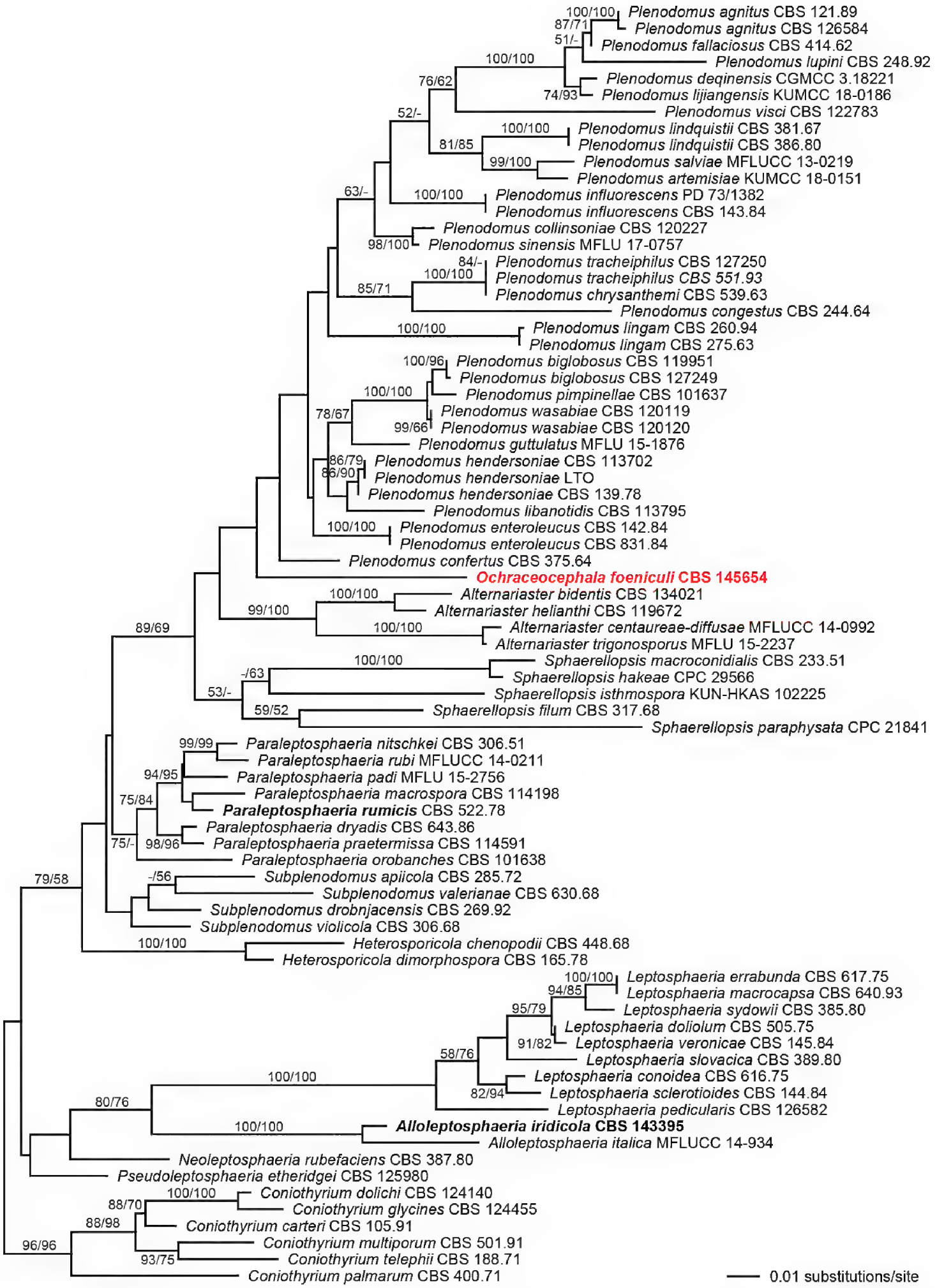
100/100 Plenodomus agnitus CBS 121.89
87/71 Plenodomus agnitus CBS 126584
51/- Plenodomus fallaciosus CBS 414.62
100/100 Plenodomus lupini CBS 248.92
76/62 Plenodomus deqinensis CGMCC 3.18221
74/93 Plenodomus lijiangensis KUMCC 18-0186
Plenodomus visci CBS 122783
52/- 100/100 Plenodomus lindquistii CBS 381.67
81/85 Plenodomus lindquistii CBS 386.80
99/100 Plenodomus salviae MFLUCC 13-0219
Plenodomus artemisiae KUMCC 18-0151
63/- 100/100 Plenodomus influorescens PD 73/1382
Plenodomus influorescens CBS 143.84
Plenodomus collinsoniae CBS 120227
98/100 Plenodomus sinensis MFLU 17-0757
84/- Plenodomus tracheiphilus CBS 127250
100/100 Plenodomus tracheiphilus CBS 551.93
85/71 Plenodomus chrysanthemi CBS 539.63
Plenodomus congestus CBS 244.64
100/100 Plenodomus lingam CBS 260.94
Plenodomus lingam CBS 275.63
100/96 Plenodomus biglobosus CBS 119951
Plenodomus biglobosus CBS 127249
100/100 Plenodomus pimpinellae CBS 101637
78/67 Plenodomus wasabiae CBS 120119
99/66 Plenodomus wasabiae CBS 120120
Plenodomus guttulatus MFLU 15-1876
86/79 Plenodomus hendersoniae CBS 113702
86/90 Plenodomus hendersoniae LTO
Plenodomus hendersoniae CBS 139.78
Plenodomus libanotidis CBS 113795
100/100 Plenodomus enteroleucus CBS 142.84
Plenodomus enteroleucus CBS 831.84
Plenodomus confertus CBS 375.64
Ochraceocephala foeniculi CBS 145654
100/100 Alternariaster bidentis CBS 134021
99/100 Alternariaster helianthi CBS 119672
89/69 100/100 Alternariaster centaureae-diffusae MFLUCC 14-0992
Alternariaster trigonosporus MFLU 15-2237
100/100 Sphaerellopsis macroconidialis CBS 233.51
-/63 Sphaerellopsis hakeae CPC 29566
53/- Sphaerellopsis isthmospora KUN-HKAS 102225
59/52 Sphaerellopsis filum CBS 317.68
Sphaerellopsis paraphysata CPC 21841
99/99 Paraleptosphaeria nitschkei CBS 306.51
Paraleptosphaeria rubi MFLUCC 14-0211
94/95 Paraleptosphaeria padi MFLU 15-2756
Paraleptosphaeria macrospora CBS 114198
75/84 Paraleptosphaeria rumicis CBS 522.78
Paraleptosphaeria dryadis CBS 643.86
75/- 98/96 Paraleptosphaeria praetermissa CBS 114591
Paraleptosphaeria orobanches CBS 101638
79/58 -/56 Subplenodomus apiicola CBS 285.72
Subplenodomus valerianae CBS 630.68
Subplenodomus drobnjacensis CBS 269.92
Subplenodomus violicola CBS 306.68
100/100 Heterosporicola chenopodii CBS 448.68
Heterosporicola dimorphospora CBS 165.78
100/100 Leptosphaeria errabunda CBS 617.75
94/85 Leptosphaeria macrocapsa CBS 640.93
95/79 Leptosphaeria sydowii CBS 385.80
Leptosphaeria doliolum CBS 505.75
91/82 Leptosphaeria veronicae CBS 145.84
58/76 Leptosphaeria slovacica CBS 389.80
100/100 Leptosphaeria conoidea CBS 616.75
82/94 Leptosphaeria sclerotioides CBS 144.84
80/76 Leptosphaeria pedicularis CBS 126582
100/100 Alloleptosphaeria iridicola CBS 143395
Alloleptosphaeria italica MFLUCC 14-934
Neoleptosphaeria rubefaciens CBS 387.80
Pseudoleptosphaeria etheridgei CBS 125980
100/100 Coniothyrium dolichi CBS 124140
88/70 Coniothyrium glycines CBS 124455
88/98 Coniothyrium carteri CBS 105.91
96/96 Coniothyrium multiporum CBS 501.91
93/75 Coniothyrium telephii CBS 188.71
Coniothyrium palmarum CBS 400.71
0.01 substitutions/site

Figure 2. Phylogram of the best ML tree (–lnL = 14211.5558) revealed by RAxML from an analysis of the combined SSU-ITS-LSU matrix of selected Leptosphaeriaceae, showing the phylogenetic position of *Ochraceocephala foeniculi* (bold red). Taxa in bold black denote new combinations proposed here. ML and MP bootstrap support above 50% are given above or below the branches.

Type species. *Ochraceocephala foeniculi* Voglmayr & Aiello.

Notes. *Ochraceocephala* is phylogenetically closely related to *Plenodomus*, from which it deviates substantially in morphology. *Plenodomus* species are characterised by pycnidial phoma-like asexual morphs, and while in two *Plenodomus* species (*P. chrysanthemi*, *P. tracheiphilus*) simple hyphomycetous, phialophora-like synanamorphs have been recorded (Boerema et al. 1994), these are very different from the complex conidiophores of the present fennel pathogen. These morphological differences, the lack of a suitable genus within Leptosphaeriaceae and its phylogenetic position therefore warrants the establishment of a new genus.

***Ochraceocephala foeniculi* Voglmayr & Aiello, sp. nov.**
MycoBank No: 833934
Figure 3

Etymology. referring to its host genus, *Foeniculum* (Apiaceae).

Colonies fast-growing, at room temperature (22 ± 1 °C) on CMD reaching 80 mm after 7 d; on MEA 38 mm after 7 d; with dull white to cream surface, upon conidiation becoming beige to olive yellow from the centre, reverse cream with greyish to dark brown centre; cottony, with abundant surface mycelium; sporulation abundant on aerial hyphae. Aerial hyphae hyaline, 2–6 µm wide. Conidiophores hyaline, produced terminally or laterally on aerial hyphae, variable in shape and branching, unbranched, loosely or densely branched up to two times; branching commonly irregularly verticillate. Phialides arising singly or in whorls of 2–5, (3.8–)5.8–13.5(–21.0) × (2.5–)3.0–4.3(–5.5) µm (n = 100), cylindrical, lageniform or ampulliform, often with a distinct collarette, producing basipetal conidial chains; polyphialides rarely present. Conidia (3.2–)3.5–6.0(–8.5) × (2.5–)3.0–4.2(–6.0) µm, l/w (1.0–)1.1–1.5(–2.1) (n = 155), hyaline to yellowish, in masses sand to olive yellow, smooth, mostly globose to subglobose, rarely broadly ellipsoid to pip-shaped, thick-walled.

Distribution. Italy (Sicily).

Host and substrate. Pathogenic on crown, roots and stems of living *Foeniculum vulgare*.

Holotype. Italy, Sicily, Catania province, Adrano, May 2017 (WU 40034); ex-holotype culture CBS 145654; ex holotype sequences MN516753 (ITS), MN516774 (LSU), MN516743 (SSU), MN520145 (*RPB2*), MN520149 (*TEF1*), MN520147 (*TUB2*).

***Alloleptosphaeria iridicola* (Crous & Denman) Voglmayr, comb. nov.**
MycoBank No: 833935

Basionym. *Subplenodomus iridicola* Crous & Denman, in Crous, Schumacher, Wingfield, Akulov, Denman, Roux, Braun, Burgess, Carnegie, Váczy, Guatimosim,

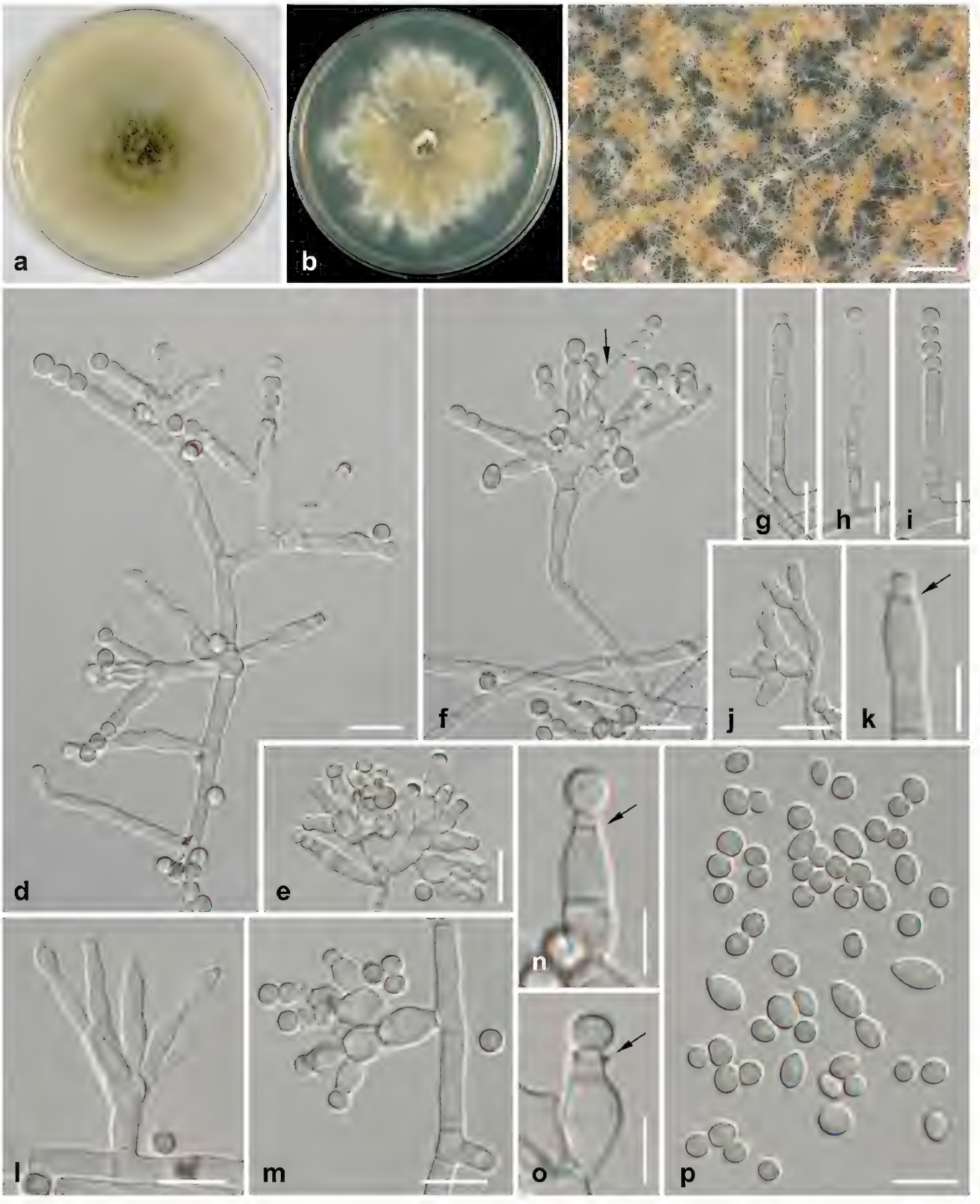

Figure 3. *Ochraceocephala foeniculi*, holotype **a** culture on CMD (7d, 22 °C) **b** culture on MEA (21d, 22 °C) **c** conidiophores on aerial hyphae producing yellowish brown conidial masses in chains **d–j, l, m** unbranched (**g–i**) and verticillately branched (**d–f, j, l, m**) conidiophores (MEA, 21d, 22 °C) with phialides; in **f** with polyphialide (arrow) **k, n, o** phialides with collarettes (arrows) and young conidia **p** conidia. All microscopic preparations from MEA (21d, 22 °C) and mounted in water. Scale bars: 200 µm (**c**); 10 µm (**d–j, l, m, p**); 5 µm (**k, n, o**).

Schwartsburd, Barreto, Hernández-Restrepo, Lombard & Groenewald, Fungal Systematics and Evolution 1: 207. 2018.

Notes. In the phylogenetic analyses (Fig. 2) *Subplenodomus iridicola* is placed remote from the other species of *Subplenodomus*, but is sister species to *Alloleptosphaeria italica* with maximum support; *S. iridicola* is therefore transferred to the genus *Alloleptosphaeria*.

***Paraleptosphaeria rumicis* (Quaedvl., Verkley & Crous) Voglmayr, comb. nov.**
MycoBank No: 833936

Basionym. *Acicuseptoria rumicis* Quaedvl., Verkley & Crous, Stud. Mycol. 75: 376 (2013).

Notes. The monotypic genus *Acicuseptoria* was described by Quaedvlieg et al. (2013) as a segregate of the polyphyletic genus *Septoria*, and it was characterised by brown, globose pycnidia with conidiophores reduced to ampulliform conidiogenous cells bearing acicular, hyaline, euseptate conidia. However, its position within the Leptosphaeriaceae remained undetermined as no other representatives of the family were included in their phylogenetic tree (Quaedvlieg et al. 2013: fig. 2). In our phylogenetic analyses (Fig. 2), *Acicuseptoria rumicis* is embedded within the genus *Paraleptosphaeria* and placed in a highly supported subclade that also contains the generic type, *P. nitschkei*. *Acicuseptoria rumicis* is therefore transferred to the genus *Paraleptosphaeria*.

Pathogenicity test

The representative isolate (CBS 145654) was pathogenic to fennel plants, and produced symptoms similar to those observed in open field after five days (Fig. 1e). The pathogen was re-isolated from the artificially inoculated plants, and identified as previously described. No symptoms were observed on control plants.

Cultivar susceptibility

In the experiments on fennel susceptibility there was always a significant effect of the cultivar on all disease parameters (PM, DI and SS) of pathogen infections ($p < 0.0001$). Otherwise, a not significant cultivar × trial effect ($p > 0.56$) was observed for parametric variables (PM and DI) in this repeated experiment (Table 5). Besides, Kendall's coefficient of concordance was 0.96 for SS data, thus indicating very high concordance between the two trials (Table 5). Therefore, the two trials were combined.

Regarding susceptibility of fennel to this phytopathogenic fungus, a great variability was detected among the tested cultivars eight days after inoculation. Comprehensively, cultivar 'Narciso' was the most susceptible since all disease parameters and

Table 5. ANOVA effects of cultivar and cultivar × trial interactions on plant mortality, disease incidence and severity of symptoms caused by *Ochraceocephala foeniculi* on inoculated young fennel plants.

Model effect	Parameter								
Factor(s)	Plant mortality (PM)[1]			Disease incidence (DI)[1]			Symptom severity (SS)[2]		
	df	*F*	*P* value	df	*F*	*P* value	χ^2	*W*	*P* value
Cultivar	5	70.6286	< 0.0001	5	33.659	< 0.0001	89.2051	…	< 0.0001
Cultivar × trial	5	0.1273	0.98475[ns]	5	0.789[ns]	0.56797[ns]	…	0.95873	0.0003

[1] *F* test of fixed effects, df = degrees of freedom, and *P* value associated to *F*; ns = not significant. [2] The χ^2 value for Kruskal-Wallis one-way analysis of variance test (cultivar) and Friedman two-way analysis of variance (cultivar × trial), respectively; *W* = Kendall's coefficient of concordance between repeated trials in the experiment.

Table 6. Compared susceptibility to crown and root rot infections of six commercial fennel cultivars.

Cultivar	Plant mortality (PM)[1]	Disease incidence (DI)[1]	Symptom severity (SS)[2]
'Narciso'	72.92 ± 2.08 a	100 a	4.15 ± 0.10 a
'Apollo'	58.33 ± 4.17 b	100 a	4.33 ± 0.17 a
'Pompeo'	45.83 ± 4.17 b	100 a	3.37 ± 0.13 b
'Aurelio'	10.42 ± 2.08 c	100 a	2.56 ± 0.06 b
'Archimede'	0.00 d	83.33 ± 4.17 b	1.94 ± 0.10 c
'Pegaso'	0.00 d	77.08 ± 2.08 b	1.48 ± 0.10 d

[1]Data derived from repeated experiment. Standard error of the mean = SEM, means are from 24 fennel young plants. Arithmetic means are presented although analysis was performed on angular transformed values. Means followed by different letters within the column are significantly different according to Fisher's least significance differences test (α = 0.05). [2] Differences among SS (0-to-5 scale) data for each treatment were analysed with Friedman two-way analysis of variance by mean rank scores ($P < 0.001$) followed by all pairwise multiple comparison with Wilcoxon.

its PM value were significantly the highest among the tested cultivars. 'Apollo' was also highly susceptible to infection by the new fennel pathogen, significantly differing only in a slightly lower PM value. 'Pompeo' displayed PM and DI values similar to those recorded for 'Apollo', but its SS score was significantly lower than in the former (Table 6). In decreasing order of susceptibility, 'Aurelio' did not significantly differ from 'Pompeo' for DI and SS values, but its PM caused by the fennel pathogen was strongly reduced. No dead seedlings (PM = 0) were recorded for both 'Archimede' and 'Pegaso', that significantly differed for DI and SS from the other remaining cultivars. Altogether, 'Pegaso' was the least susceptible cultivar to fungal infection since it showed the lowest values of disease severity.

Discussion

In the present study, 32 fungal isolates were recovered from symptomatic fennel plants in Sicily over a 2-year period. Disease symptoms were observed in three farms, and included necrotic lesions and crown and root rot on three different cultivars. The fungal species obtained from symptomatic tissues was identified based on morphological characters and molecular phylogenetic analyses of an ITS-LSU-SSU rDNA matrix, resulting in the description of the fennel pathogen as a new genus and species, *Ochraceocephala foeniculi*.

In the phylogenetic analyses, *O. foeniculi* was revealed as sister group of *Plenodomus*; however, without significant support (Fig. 2). As commonly observed with ITS-LSU-SSU rDNA data, support of many backbone nodes is low or absent, and additional protein-coding markers like *RPB2*, *TEF1* and *TUB2* are necessary for an improved phylogenetic resolution of genera and families in Pleosporales (Voglmayr and Jaklitsch 2017; Jaklitsch et al. 2018). Although we sequenced *RPB2*, *TEF1*, and *TUB2* for *O. foeniculi*, it was currently not feasible to perform multi-gene analyses due to insufficient sequence data for most species of Leptosphaeriaceae, in particular for *Plenodomus*. However, we consider the phylogenetic and morphological evidence conclusive for establishing the new genus *Ochraceocephala*. Also the generic transfer of *Subplenodomus iridicola* to *Alloleptosphaeria* is well substantiated, considering its highly supported phylogenetic position as sister species of *Alloleptosphaeria italica*, remote from the generic type (*S. violicola*) and other species of *Subplenodomus* (Fig. 2). In the phylogenetic analyses of the LSU rDNA matrix of Crous et al. (2018: fig. 1), only few taxa of Leptosphaeriaceae were included, and the phylogenetic position of *S. iridicola* remained inconclusive due to low resolution; however, also in their analyses it was placed remote from the generic type, *S. violicola*. In addition, they did not include its closest relative, *Alloleptosphaeria italica*, although it was mentioned as the closest match of an ITS BLAST search (Crous et al. 2018). No asexual morph is known for *A. italica* (Dayarathne et al. 2015), but the ascomata, asci and ascospores of *A. iridicola* and *A. italica* share many traits. Our phylogenetic analyses also showed that *Acicuseptoria rumicis* should be included within *Paraleptosphaeria* (Fig. 2). Although it was correctly placed within Leptosphaeriaceae by Quaedvlieg et al. (2013), its position within the family remained undetermined as no other representatives of the family were included in their phylogenetic analyses. As for most other species of *Paraleptosphaeria* no asexual morphs are known, no comprehensive morphological comparison can currently be made with *P. rumicis*.

Within Leptosphaeriaceae, *O. foeniculi* is remarkable and unique by its complex hyphomycetous asexual morph composed of branched conidiophores with phialidic conidiation and conidia produced in basipetal chains. Asexual morphs in Leptosphaeriaceae are typically coelomycetous and phoma-like, which is also the case in the closest relative of *Ochraceocephala*, *Plenodomus* (Gruyter et al. 2013). Another genus of Leptosphaeriaceae with a hyphomycetous asexual morph is *Alternariaster*, which, however, differs significantly by tretic condiogenous cells forming large, brown, septate conidia not produced in chains (Simmons 2007; Alves et al. 2013). Therefore, the unique morphology in combination with an isolated phylogenetic position within Leptosphaeriaceae warrant the establishment of a new genus.

Other fungal species belonging to Leptosphaeriaceae, as well as the closely related Didymellaceae (Odstrčilová et al. 2002; Shaker and Alhamadany 2015) have been reported worldwide in fennel crops. In Italy, crown rot of fennel caused by *Didymella glomerata* (syn. *Phoma glomerata*) was recorded from southern Italy (Lahoz et al. 2007). As confirmed in the pathogenicity tests, *O. foeniculi* caused symptoms on artificially inoculated plants of the same cultivar and, moreover, also on different fennel cultivars

that showed some variability in disease susceptibility. To this regard, it is noteworthy that this study also represents a preliminary evaluation of fennel germplasm according to their susceptibility to this new disease. Although these data should be confirmed by additional investigations, this study might provide very useful information for local farmers and technicians. The determination of the extent of susceptibility to *O. foeniculi* is a starting point for evaluating the tolerance of commercial fennel cultivars to this disease under different agronomic and phytosanitary conditions.

On the basis of the disease incidence and severity observed in the field, we believe that this disease represents a serious threat to fennel crop in Sicily and may become a major problem also to other areas of fennel production if accidentally introduced. Moreover, infected soil could represent an inoculum source for this fungus. Further studies are needed to examine the life cycle of *O. foeniculi* and to ascertain the cardinal temperatures of the fungus for successful infection since this pathogen is well established in this representative fennel production area. This information is required for the setup and timing of sustainable approaches for soil disinfection, including solarization and/or fumigation at low rates, to reduce the level of the primary inoculum in the soil and hence the disease amount, like successfully applied for other soilborne plant pathogens (Vitale et al. 2013; Aiello et al. 2018).

Although not always conclusive, soil disinfestation and host resistance can be considered environmentally friendly means to be included within integrated pest management (IPM) strategies against crown rot caused by *O. foeniculi* in order to minimize the number and intensity of fungicide applications.

Acknowledgements

This research was supported by Research Project 2016–2018 "Monitoraggio, caratterizzazione e controllo sostenibile di microrganismi e artropodi di interesse agrario" WP2-5A722192134.

References

Aiello D, Vitale A, Alfenas RS, Alfenas AC, Cirvilleri G, Polizzi G (2018) Effects of sublabeled rates of dazomet and metam-sodium applied under low-permeability films on *Calonectria* microsclerotia survival. Plant Disease 102: 782–789. https://doi.org/10.1094/PDIS-06-16-0801-RE

Alves JL, Woudenberg JH, Duarte LL, Crous PW, Barreto RW (2013) Reappraisal of the genus *Alternariaster* (Dothideomycetes). Persoonia 31: 77–85. https://doi.org/10.3767/003158513X669030

Ariyawansa HA, Phukhamsakda C, Thambugala KM, Bulgakov TS, Wanasinghe DN, Perera RH, Mapook A, Camporesi E, Kang JC, Jones EG, Bahkali AH, Jayasiri SC, Hyde KD,

Liu ZY, Bhat JD (2015) Revision and phylogeny of Leptosphaeriaceae. Fungal Diversity 74: 19–51. https://doi.org/10.1007/s13225-015-0349-2

Boerema GH, de Gruyter J, van Kesteren HA (1994) Contributions towards a monograph of *Phoma* (*Coelomycetes*) – III. 1. Section *Plenodomus*: Taxa often with a *Leptosphaeria* teleomorph. Persoonia 15: 431–487.

Cacciola SO, Pane A, Cooke DEL, Raudino F, Magnano di San Lio G (2006) First report of brown rot and wilt of fennel caused by *Phytophthora megasperma* in Italy. Plant Disease 90: 110. https://doi.org/10.1094/PD-90-0110A

Carbone I, Kohn LM (1999) A method for designing primer sets for speciation studies in filamentous ascomycetes. Mycologia 91: 553–556. https://doi.org/10.2307/3761358

Choi IY, Hong SH, Cho SE, Park JH, Shin HD (2015) First report of powdery mildew caused by *Erysiphe heraclei* on fennel (*Foeniculum vulgare*) in Korea. Plant Disease 99: 1185. https://doi.org/10.1094/PDIS-02-15-0147-PDN

Choi IY, Kim JH, Kim BS, Park MJ, Shin HD (2016) First report of Sclerotinia stem rot of fennel caused by *Sclerotinia sclerotiorum* in Korea. Plant Disease 100: 223. https://doi.org/10.1094/PDIS-05-15-0512-PDN

Crous PW, Schumacher RK, Wingfield MJ, Akulov A, Denman S, Roux J, Braun U, Burgess TI, Carnegie AJ, Váczy KZ, Guatimosim E, Schwartsburd PB, Barreto RW, Hernández-Restrepo M, Lombard L, Groenewald JZ (2018) New and interesting fungi. 1. Fungal Systematics and Evolution 1: 169–215. https://doi.org/10.3114/fuse.2018.01.08

D'Amico M, Frisullo S, Cirulli M 2008. Endophytic fungi occurring in fennel, lettuce, chicory, and celery-commercial crops in southern Italy. Mycological Research 112: 100. https://doi.org/10.1016/j.mycres.2007.11.007

Dayarathne MC, Phookamsak R, Ariyawansa HA, Jones EBG, Camporesi E, Hyde KD (2015) Phylogenetic and morphological appraisal of *Leptosphaeria italica* sp. nov. (Leptosphaeriaceae, Pleosporales) from Italy. Mycosphere 6: 634–642. https://doi.org/10.5943/mycosphere/6/5/13

Ghoneem KM, Saber WA, Elwakil MA (2009) Alkaline seed bed an innovative technique for manifesting *Verticillium dahliae* on fennel seeds. Plant Pathology Journal 8: 22–26. https://doi.org/10.3923/ppj.2009.22.26

Glass NL, Donaldson GC (1995) Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. Applied and Environmental Microbiology 61: 1323–1330. https://doi.org/10.1128/AEM.61.4.1323-1330.1995

Gruyter Jde, Woudenberg JH, Aveskamp MM, Verkley GJ, Groenewald JZ, Crous PW (2013) Redisposition of phoma-like anamorphs in Pleosporales. Studies in Mycology 75: 1–36. https://doi.org/10.3114/sim0004

Hoog GS de, Gerrits van den Ende AHG (1998) Molecular diagnostics of clinical strains of filamentous basidiomycetes. Mycoses 41: 183–189. https://doi.org/10.1111/j.1439-0507.1998.tb00321.x

ISTAT (Istituto Nazionale di Statistica) (2018) Ortive: finocchio in pien'aria. http://dati.istat.it [Accessed on: 2019-09-16]

Jaklitsch, WM (2009) European species of *Hypocrea* Part I. The green-spored species. Studies in Mycology 63: 1–91. https://doi.org/10.3114/sim.2009.63.01

Jaklitsch WM, Checa J, Blanco MN, Olariaga I, Tello S, Voglmayr H (2018) A preliminary account of the Cucurbitariaceae. Studies in Mycology 90: 71–118. https://doi.org/10.1016/j.simyco.2017.11.002

Jaklitsch WM, Komon M, Kubicek CP, Druzhinina IS (2005) *Hypocrea voglmayrii* sp. nov. from the Austrian Alps represents a new phylogenetic clade in *Hypocrea*/*Trichoderma*. Mycologia 97: 1365–1378. https://doi.org/10.1080/15572536.2006.11832743

Koike ST, Tompkins DV, Martin F, Ramon ML (2015) First report of Pythium root rot of fennel in California caused by *Pythium sulcatum*. Plant Disease 99: 1645. https://doi.org/10.1094/PDIS-03-15-0288-PDN

Khare MN, Tiwari SP, Sharma YK (2014) Disease problems in fennel (*Foeniculum vulgare* Mill) and fenugreek (*Trigonella foenum graceum* L.) cultivation and their management for production of quality pathogen free seeds. International Journal of Seed Spices 4: 11–17.

Lahoz E, Caiazzo R, Fanigliulo A, Comes S, Crescenzi A (2007) *Phoma glomerata* as causal agent of crown rot disease of fennel in southern Italy. Communications in Agricultural and Applied Biological Sciences 72: 875–878.

Liu YL, Whelen S, Hall BD (1999) Phylogenetic relationships among ascomycetes: evidence from an RNA polymerase II subunit. Molecular Biology and Evolution 16: 1799–1808. https://doi.org/10.1093/oxfordjournals.molbev.a026092

O'Donnell K, Cigelnik E (1997) Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus *Fusarium* are nonorthologous. Molecular Phylogenetics and Evolution 7: 103–116. https://doi.org/10.1006/mpev.1996.0376

Odstrčilová L, Ondřej M, Kocourková B, Růžičková G (2002) Monitoring of incidence and determination of fungi on caraway, fennel, coriander and anise, consideration of disease importance and possibility of chemical protection. Plant Protection Science 38: 340–343. https://doi.org/10.17221/10485-PPS

Phookamsak R, Hyde KD, Jeewon R, Bhat DJ, Jones EBG, Maharachchikumbura SSN, Raspé O, Karunarathna SC, Wanasinghe DN, Hongsanan S, Doilom M, Tennakoon DS, Machado AR, Firmino AL, Ghosh A, Karunarathna A, Mešić A, Dutta AK, Thongbai B, Devadatha B, Norphanphoun C, Senwanna C, Wei D, Pem Di, Ackah FK, Wang GN, Jiang HB, Madrid H, Lee HB, Goonasekara ID, Manawasinghe IS, Kušan I, Cano J, Gené J, Li J, Das K, Acharya K, Raj KNA, Latha KPD, Chethana KWT, He MQ, Dueñas M, Jadan M, Martín MP, Samarakoon MC, Dayarathne MC, Raza M, Park MS, Telleria MT, Chaiwan N, Matočec N, de Silva NI, Pereira OL, Singh PN, Manimohan P, Uniyal P, Shang QJ, Bhatt RP, Perera RH, Alvarenga RLM, Nogal-Prata S, Singh SK, Vadthanarat S, Oh SY, Huang SK, Rana S, Konta S, Paloi S, Jayasiri SC, Jeon SJ, Mehmood T, Gibertoni TB, Nguyen TTT, Singh U, Thiyagaraja V, Sarma VV, Dong W, Yu XD, Lu YZ, Lim YW, Chen Y, Tkalčec Z, Zhang ZF, Luo ZL, Daranagama DA, Thambugala KM, Tibpromma S, Camporesi E, Bulgakov TS, Dissanayake AJ, Senanayake IC, Dai DQ, Tang LZ, Khan S, Zhang H, Promputtha I, Cai L, Chomnunti P, Zhao RL, Lumyong S, Boonmee S, Wen T-C, Mortimer PE, Xu J (2019) Fungal diversity notes 929–1035: taxonomic and phylogenetic contributions on genera and species of fungi. Fungal Diversity 95: 1–273. https://doi.org/10.1007/s13225-019-00421-w

Quaedvlieg W, Verkley GJ, Shin HD, Barreto RW, Alfenas AC, Swart WJ, Groenewald JZ, Crous PW (2013) Sizing up *Septoria*. Studies in Mycology 75: 307–390. https://doi.org/10.3114/sim0017

Rodeva R, Gabler J (2011) Umbel browning and stem necrosis: a new disease of fennel in Bulgaria. Journal of Phytopathology 159: 184–187. https://doi.org/10.1111/j.1439-0434.2010.01728.x

Shaker GA, Alhamadany HS (2015) Isolation and identification of fungi which infect fennel *Foeniculum vulgare* Mill. and its impact as antifungal agent. Iraq Natural History Research Center and Museum 13: 31–38.

Silvestro D, Michalak I (2012) raxmlGUI: a graphical front-end for RAxML. Organisms Diversity and Evolution 12: 335–337. https://doi.org/10.1007/s13127-011-0056-0

Simmons EG (2007) *Alternaria*: An Identification Manual. CBS Biodiversity Series 6. Centraalbureau voor Schimmelcultures, Utrecht, Netherlands, 775 pp.

Stamatakis E (2006) RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22: 2688–2690. https://doi.org/10.1093/bioinformatics/btl446

Sung GH, Sung JM, Hywel-Jones NL, Spatafora JW (2007) A multi-gene phylogeny of Clavicipitaceae (Ascomycota, Fungi): Identification of localized incongruence using a combinational bootstrap. Molecular Phylogenetics and Evolution 44: 1204–1223. https://doi.org/10.1016/j.ympev.2007.03.011

Swofford DL (2002) PAUP* 4.0b10: phylogenetic analysis using parsimony (*and other methods). Sinauer Associates, Sunderland, Massachusetts. https://doi.org/10.1111/j.0014-3820.2002.tb00191.x

Taubenrauch K, Gabler J, Hau B (2008) Mykologische Untersuchung von *Mycosphaerella anethi* an Fenchel (*Foeniculum vulgare* Mill.). Mitteilungen aus dem Julius Kühn-Institut 417: 387–388.

Vilgalys R, Hester M (1990) Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. Journal of Bacteriology 172: 4238-4246. https://doi.org/10.1128/jb.172.8.4238-4246.1990

Vitale A, Castello I, D'Emilio A, Mazzarella R, Perrone G, Epifani S, Polizzi G (2013) Short-term effects of soil solarization in suppressing *Calonectria* microsclerotia. Plant and Soil 368: 603–617. https://doi.org/10.1007/sl1104-012-1544-5

Voglmayr H, Jaklitsch WM (2017) *Corynespora*, *Exosporium* and *Helminthosporium* revisited – new species and generic reclassification. Studies in Mycology 87: 43–76. https://doi.org/10.1016/j.simyco.2017.05.001

Voglmayr H, Rossman AY, Castlebury LA, Jaklitsch WM (2012) Multigene phylogeny and taxonomy of the genus *Melanconiella* (Diaporthales). Fungal Diversity 57: 1–44. https://doi.org/10.1007/s13225-012-0175-8

White TJ, Bruns TD, Lee SB, Taylor JW (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (Eds) PCR Protocols: a Guide to Methods and Applications. Academic Press, New York, 315–322. https://doi.org/10.1016/B978-0-12-372180-8.50042-1